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Targeted Enzyme Discovery in Feedstock-adapted Microbial Communities

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Efficient saccharification of pre-treated feedstocks is essential to produce inexpensive biofuels derived from biomass. The enzyme cocktails used for this step need to be robust and able to withstand inhibitors produced during pretreatment. To identify enzymes suitable for saccharification on an industrial scale, we have focused on adapting microbial communities to specific feedstocks and pretreatment conditions (i.e. high temperatures, ionic liquid contamination, etc.), followed by functional characterization of secreted glycosyl hydrolases. To select for thermophilic enzymes, microbial communities derived from compost were adapted to biomass feedstocks at 60°C. Phylogenetic profiling of these communities show that each consists of a simple mixed consortia with just two or three species in high abundance. The secretomes obtained from these consortia have biomass-deconstructing enzymatic activity that is both thermostable and active in high concentrations of ionic liquid, two highly desirable characteristics for industrial enzymes. Zymography was used to investigate the complement of glycosyl hydrolase enzymes expressed by the consortia, detecting at least a dozen active enzymes for multiple polysaccharide substrates. Currently, proteins within individual zymogram bands are being studied by MS-based proteomics. Candidate glycosyl hydrolases will be identified by comparing measured peptides masses to predicted protein sequences from Carbohydrate Active enZYme (CAZy) database and genome sequences of reference organisms related to consortial members. Future work will utilize metagenomic and single-cell genomic sequencing to document comprehensively the glycosyl hydrolases secreted by these highly active microbial communities. Cataloguing the glycosyl hydrolases in these secretomes will enable us to design thermophilic enzyme cocktails for biomass deconstruction that function under the conditions required for industrial conversion of biomass to biofuels.

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